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The Use of Semi-Automated EBV IgG Avidity Determination for the Diagnosis of Infectious Mononucleosis

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The diagnosis of acute Epstein-Barr virus (EBV) infection is based frequently on the combination of positive viral capsid antigen (VCA) IgM antibodies and negative EB viral nuclear antigen 1 (EBNA-1) IgG antibodies. However, both VCA IgM and EBNA-1 IgG can provide false positive and false negative results. Therefore, situations in which the EBV serology remains unclear are not uncommon. Determination of EBV IgG avidity can clarify the EBV status in these patients. So far, mainly immunofluorescence assays have been used for this purpose. These tests are laborious, their evaluation is subjective, and automation is difficult. Therefore, two commercially available microtiter plate enzyme immunoassays (EIA) were compared for their usefulness for semi-automated EBV IgG avidity determination. One assay is based on a mixture of EBV antigens, the other assay uses a synthetic peptide of the VCA-complex. Patient sera of confirmed acute and past EBV infections were tested for avidity by both assays. The results with the antigen mixture assay proved to be highly sensitive (100%) and specific (100%). Avidity index calculations on the basis of one-point-quantification titers gave better results than calculations using OD values. Determination of EBV IgG avidity by the peptide assay was complicated by the fact that it was less sensitive than the antigen mixture assay for IgG detection in acute EBV infections. On the other hand, about 30% of the samples had to be retested with the peptide assay in a higher dilution because the IgG units in initial testing fell outside the range covered by the standard curve. Using OD values of the peptide EIA, the sensitivity was 99% but the specificity of detection of acute EBV infections was only 86%. Thus, while the peptide EBV avidity assay is unsuitable as a confirmatory assay, avidity testing with the antigen mixture assay is a useful tool to resolve equivocal EBV serologies. Avidity assays on the basis of EIA can be automated which should lead to wider use of this methodology. ***J. Med. Virol. 54:145–153, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: antibody avidity; avidity index; antibody maturation; Epstein-Barr virus; Epstein-Barr virus serology; acute infection

INTRODUCTION

The clinical presentation of acute Epstein-Barr-virus (EBV) infection is very diverse. While often asymptomatic in childhood, it presents typically as mononucleosis in young adults. Main symptoms usually include pharyngitis, lymphadenopathy and hepatosplenomegaly, but many other organs may also be affected. Hematologic, neurologic, cardiac or pulmonary complications may even predominate the clinical picture [Okano et al., 1988]. Thus, a situation commonly encountered in the diagnostic laboratory is to confirm or exclude an acute EBV infection. Many diagnostic tests and strategies are used for this purpose. Frequently, the diagnosis is based on determination of antibodies against Epstein-Barr viral nuclear antigen 1 (EBNA-1) and viral capsid antigen (VCA). Whereas VCA IgM is usually found at the onset of mononucleosis symptoms, EBNA-1 antibodies become detectable only months after a primary EBV infection. Therefore, the constellation of positive VCA IgM and negative EBNA-1 antibodies strongly supports the diagnosis of an acute EBV infection, while negative VCA IgM and positive EBNA-1 antibodies argue for a past infection [Henle et al., 1974; Henle et al., 1987; Okano et al., 1988; Linde, 1992].

However, in practice the distinction is often much less clear. During acute EBV infection, IgM antibodies may be only weakly or not detectable [Nikoskelainen et al., 1974; Evans et al., 1975; Fleisher et al., 1979;

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Schillinger et al., 1993], especially in immunocompromised patients such as HIV infected individuals or organ transplant recipients [Gray, 1995]. On the other hand, IgM antibodies may persist over months after an acute infection, or may become positive again during EBV reactivation [Schmitz et al., 1972; Sumaya, 1977]. When enzyme immunoassays (EIA) are used for EBV IgM detection, false positive or false negative results are observed frequently [Wiedbrauk and Bassin, 1993; Weber et al., 1996]. Similar problems exist with EBNA-1 antibodies: in some patients with a past EBV infection, they may remain below the detection limit [Lamy et al., 1982; Horwitz et al., 1985; Kampmann et al., 1993]. In addition, immunosuppressed patients are frequently unable to mount an EBNA-1 antibody response [Lange et al., 1978; Henle and Henle, 1981; Miller et al., 1987; Vetter et al., 1994]. Thus, a differentiation of acute from reactivated or latent infection may be very difficult in these patients.

At first in the acute phase of an infection, low avidity antibodies are secreted and, as the immune response matures over the course of several weeks, high avidity antibodies can finally be detected [Eisen and Siskind, 1964; Werblin et al., 1973; Inouye et al., 1984; Kocks and Rajewsky, 1988]. In this context, the assessment of antibody avidity can be used to estimate the time at which primary infection occured. Classically, avidity indices have been derived from titration of serum samples with and without urea washing steps. To overcome the considerable expense and time required for these titration curves, simplified avidity tests based on single point determinations using EIAs have been described [Hedman and Rousseau, 1989; Blackburn et al., 1991; Gray, 1995; Gassmann and Bauer, 1997].

Avidity testing of EBV IgG has been employed successfully in order to diagnose cases of acute EBV infections and to resolve cases of otherwise equivocal EBV serology. Most of these studies have used immunofluorescence assays (IFA) which remain the "gold standard" in EBV serology [Gray and Wreghitt, 1989; de Ory et al., 1993; Andersson et al., 1994; Vetter et al., 1994]. However, these techniques are laborious because two titration curves are required and the evaluation of IFA slides tends to be subjective. Gray [1995] has shown the usefulness of a VCA IgG EIA to detect low avidity antibodies in 28 immunocompetent and 7 immunocompromised patients with recent EBV infection. Data on the time points after disease onset were not provided in this study.

In an attempt to simplify and automate EBV IgG avidity tests, it was demonstrated previously that cases of acute and past EBV infections can be clearly differentiated by semi-automated EBV IgG avidity testing based on single-point-determinations using a commercially available EIA [Schubert et al., 1996]. The results were superior to IFA avidity, but the IFA was more sensitive for IgG detection in acute EBV infections. For several acute infection sera, an avidity index could not be determined by EIA because EBV IgG antibodies were negative with or even without a urea washing step. While the IFA was based on detection of IgG antibodies against EBV VCA, the EIA microtiter plate was coated with a mixture of EBV antigens. In this study, it was investigated whether a VCA based EIA allows avidity determination with increased sensitivity for IgG detection in early EBV infections. For this purpose, a commercially available VCA EIA was compared with the previously used antigen mixture EIA. In addition, the use of raw optical density (OD) values and of quantified arbitrary EBV IgG units for single point avidity determinations were evaluated.

MATERIALS AND METHODS

Samples

Two groups of patients were tested for EBV IgG avidity. Group 1 consisted of 92 serum samples of 60 patients with acute EBV infection and known disease onset. Group 2 consisted of 53 serum samples of 52 patients with past EBV infection. The samples had been sent to our department for routine serologic analysis and had been stored at −20°C until testing. For group 1, the diagnosis of acute EBV infection was based on positive VCA IgM (EBV VCA IgM Elisa, Ortho Diagnostic Systems, Neckargemünd, Germany) or EBV IgM (Enzygnost Anti-EBV/IgM, Behring Diagnostics, Marburg, Germany), negative EBNA-1 antibodies (in house anticomplement IFA using P3HR1 cells according to Kampmann et al. [1993]) and the presence of clinical symptoms suggestive of mononucleosis. Samples were included in group 2 if the presence of EBV antibodies had been recorded in previous samples of the same patient at least 6 months earlier. Before testing for EBV IgG avidity, the samples were coded and randomised.

Avidity Determination

For EBV IgG avidity testing two different microtiter plate EIAs were used (Enzygnost Anti-EBV/IgG, Behring Diagnostics, Marburg, Germany; and ETI-VCA-G, Sorin Biomedica, Düsseldorf, Germany). The Behring microtiter plates were coated with an antigen mixture that contains VCA, EBNA and EA. Strips with control antigen were also included. The Sorin assay used a synthetic p18-peptide of the VCA complex as antigen. The tests were carried out according to the instructions of the manufacturer with the following modifications. Each of the appropriately diluted serum samples was placed in two antigen coated microtiter plate wells. After 60 min of incubation at 37°C, one well was washed with the washing buffer provided according to the instructions, the other well was washed three times with a solution of urea in the supplied washing buffer (for urea concentration, see results) and once with washing buffer only. The subsequent steps were carried out according to the manufacturer's instructions in an automated fashion using the Behring Elisa Processor III (BEP III; Behring Diagnostics, Marburg, Germany). Control samples of acute and past EBV infections were included in each run. EBV IgG antibodies were quantified in arbitrary units (U) according to a one-point-quantification method (alpha-method, Be-

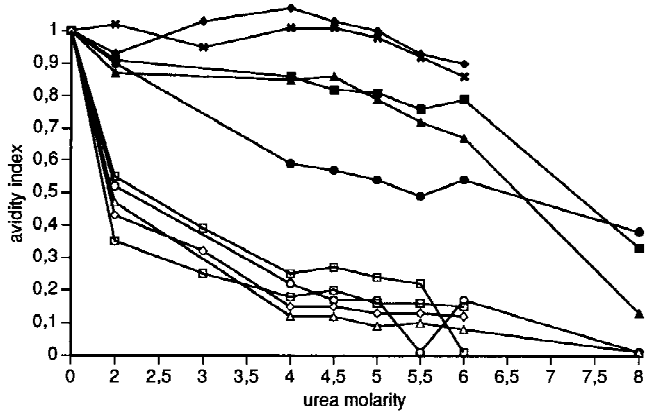

Fig. 1. Optimization of urea concentration for the peptide avidity assay. The EBV IgG avidity of five acute infection sera (open symbols) and five past infection sera (closed symbols) was tested with different urea concentrations.

hring) or by a standard curve (Sorin). If the IgG units were higher than the upper limit of the assay, the serum sample was diluted tenfold and then retested. For each serum sample, the avidity index was calculated as the quotient of EBV IgG units with the urea washing step and EBV IgG units without the urea washing step (unit avidity index). Avidity indices were also calculated using the raw OD values instead of the quantified EBV IgG values (OD avidity index). For initial evaluation, an avidity index of less than 0.5 was considered to be indicative of acute or recent infection [Gray, 1995]. If in initial testing EBV antibodies from samples of the acute or past infection group were found to have high (>0.5) or low avidity (<0.5), respectively, the samples were retested twice and the mean value of all three determinations was used subsequently.

RESULTS

Urea Concentration

For both EIAs, the optimal urea concentration in the first washing step was determined by testing several serum samples of acute and past EBV infections with varying urea concentrations. For the antigen mixture EIA, it had been shown previously that good results are obtained with a urea concentration of 5.0 M [Schubert et al., 1996]. Further optimization revealed that a 4.7 M concentration is slightly superior to 5.0 M (data not shown). Therefore, a 4.7 M urea solution was used for avidity testing with the antigen mixture EIA. For the peptide EIA, a 5.0 M urea concentration was found to give the best separation of acute and past infections in initial experiments (Fig. 1). This concentration was used for all further tests.

Avidity Based on IgG Units of the Antigen Mixture EIA

In order to compare the performance of the two microtiter plate EIAs in EBV IgG avidity analysis, 92 serum samples of patients with confirmed acute infection (group 1) and 53 serum samples of patients with past EBV infection (group 2) were tested for EBV IgG avidity with the antigen mixture and the peptide EIA. For the antigen mixture EIA, avidity indices were initially calculated based on EBV IgG units derived from single point quantification according to the test instructions. In Figure 2a–c, avidity results of the acute and past infection samples are shown in relation to the EBV IgG units without urea treatment and in relation to the days after onset of disease. As can be seen, there was a clear separation of the two groups with the antigen mixture assay. All past infection samples had an avidity index of 0.6 or higher (Fig. 2b). In group 1, all but one sample had an avidity index lower than the cut-off value of 0.5, thus confirming an acute or recent infection. For one sample of this group taken more than 10 weeks after disease onset, the avidity index was 0.66 (Fig. 2c). Serum samples of the same patient from day 5 and 29 after disease onset were also included in the study. Avidity results for these samples were 0.09 and 0.46, respectively.

In group 1 and group 2, there was no correlation between IgG units without urea treatment and the avidity index (Fig. 2a,b). For some acute infection samples, an avidity index could not be determined because IgG units were below the detection limit. This was the case for 10 sera even without urea treatment, and 29 additional sera became negative with urea washing step. An upper limit for the avidity index was calculated in these cases, when IgG units were positive without, but negative with urea washing step. For example, if IgG was 50 U without and negative (<25 U) with urea treatment, the upper limit of the avidity index is 0.5. If lower than 0.5, low antibody avidity can be assumed even without knowing the exact avidity index. Therefore, these values are shown in Figure 2c as crosses. If the upper limit was higher than 0.5, no judgement on antibody avidity could be made and therefore, these samples were not included in Figure 2c and in the statistical analysis. Overall, both the sensitivity and the specificity of the antigen mixture avidity test based on IgG units as shown in Figure 2c were 100% for the remaining samples.

Avidity Based on OD Values of the Antigen Mixture EIA

To overcome the problem of negative IgG units with urea treatment, all indices were recalculated using the raw OD values of the antigen mixture EIA in order to study if avidity index determination based on OD values is sufficient to differentiate between acute and past infections. The corresponding OD avidity results are shown in Figure 2d–f and Table 1. According to the instructions of the manufacturer, samples with OD values lower than 0.1 without urea treatment are negative for EBV IgG. Therefore, avidity indices calculated in these cases were not regarded as meaningful (vertical lines in Fig. 2d and 2e). They were excluded from the statistical analysis and are not shown in Figure 2f. For the past infection samples, OD avidity results (Fig. 2e) are comparable to unit avidity results (Fig. 2b) and

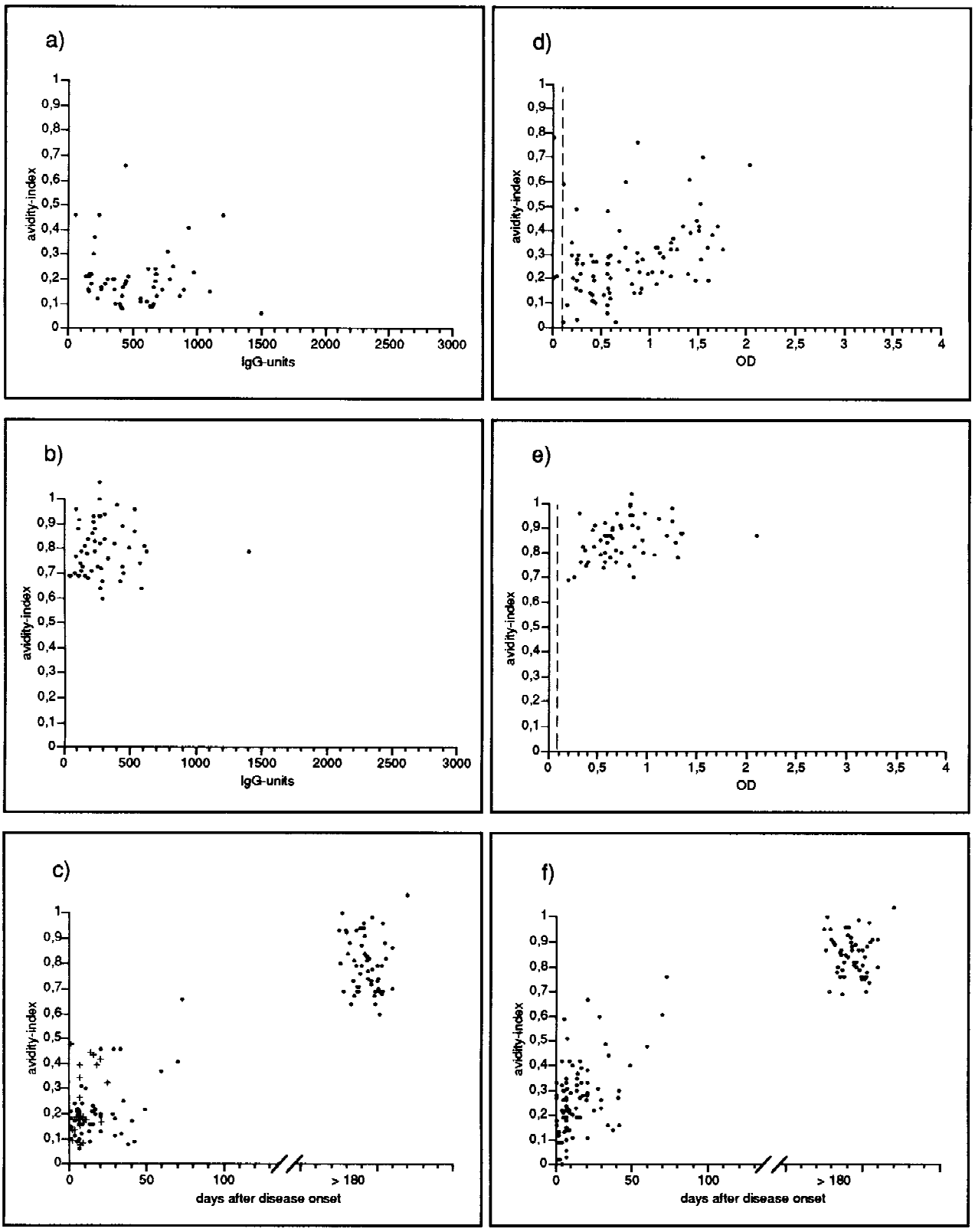

Fig. 2. Avidity determination with the antigen mixture EIA. IgG units without urea treatment and avidity indices of (a) acute infection samples and (b) past infection samples. (c) Time after onset of clinical symptoms and avidity indices based on IgG units; upper limit values for avidity indices are shown as crosses. OD values without urea treatment and avidity indices of (d) acute infection samples and (e) past infection samples. (f) Time after onset of clinical symptoms and avidity indices based on OD values.

TABLE I. Mean Optical Density (OD) Values, Mean Units and Mean Avidity Indices of the Different Avidity Determinations

	Acute infections					Past infections				
	Mean OD values and mean units			Avidity index		Mean OD values and mean units			Avidity index	
Test	No. of samples	With urea	Without urea	Mean value	Standard deviation	No. of samples	With urea	Without urea	Mean value	Standard deviation
Antigen mixture EIA units	53	103	519	0.20	0.11	53	236	296	0.80	0.11
Antigen mixture EIA OD values	83	0.25	0.81	0.27	0.15	53	0.66	0.76	0.85	0.08
Peptide EIA units	25	140	627	0.28	0.15	51	463	647	0.72	0.21
Peptide EIA OD values	79	0.22	1.23	0.21	0.12	51	0.91	1.26	0.72	0.20

were 100% specific. While the use of OD values allowed the calculation of defined avidity indices in the acute infection cases where IgG units were negative with urea treatment, the avidity indices were more widely scattered than the indices based on IgG units (2c and 2f). Consequently, the sensitivity for detection of acute EBV infection was only 93%. From the mean values and standard deviations for the different avidity index calculations (Table 1) it is also apparent that the difference between acute and past infections was slightly less distinct when using OD values instead of IgG units with the antigen mixture assay.

Avidity Based on IgG Units of the Peptide EIA

The situation was different when the peptide EIA was used. In the initial testing using the starting dilution recommended by the manufacturers (1:101), the OD values of 16 acute infection samples and 33 past infection samples were too high and fell outside the range covered by the standard curve (>170 U). Therefore, IgG units could not be calculated and the samples had to be retested at higher dilutions. On the other hand, determination of avidity indices based on IgG units was complicated by the fact that 15 acute infection samples were below the cut-off value (20 U) of the peptide EIA even without urea washing step. Fifty-two additional acute infection and two past infection samples were negative for VCA IgG with urea washing step. Thus, the peptide EIA was clearly less sensitive than the antigen mixture EIA and as a result, 67 avidity indices could not be calculated based on IgG units of the peptide assay.

Of the remaining samples (Fig. 3a–c), two sera of acute infections were found to have high EBV IgG avidity (>0.5) and would have been falsely classified as past infections. Similarly, seven samples of the past infection group had a low avidity index (<0.5) and would have been falsely classified as acute or recent infections. All samples with false high or low avidity index were retested twice and the results were reproducible in all cases. As for the antigen mixture assay, the upper limit values for the avidity indices were calculated wherever possible and are shown as crosses in Figure 3c if lower than 0.5. These cases included, the sensitivity and specificity of avidity results using IgG units of the peptide assay was 97% and 87%, respectively.

Avidity Based on OD Values of the Peptide EIA

Because of the lack of sensitivity for IgG determination in units by the peptide EIA, avidity indices were again calculated from OD values. These results are shown in Figure 3d–f and Table 1. Because of the distribution of avidity indices in relation to the OD values, it was necessary to exclude samples with an OD of lower than 0.2 from the analysis (vertical lines in Fig. 3d, 3e). For the remaining samples, the avidity results using OD values had a sensitivity of 99% and a specificity of 86% and were therefore comparable to the results based on IgG units.

DISCUSSION

In the past, serologic diagnosis based on antibody avidity has been used successfully for several viral and non-viral pathogens [Gray and Wreghitt, 1989; Hedman et al., 1989; Hedman and Rousseau, 1989; Blackburn et al., 1991; Hedman et al., 1991; Kangro et al., 1991; Meurman et al., 1992; Ward et al., 1993; Ward et al., 1994; Soderlund et al., 1995; Tuokko, 1995; Gassmann and Bauer, 1997]. Avidity tests serve to confirm or exclude an acute infection with the specific pathogen tested. EBV serology is complex and many different markers are in use. Avidity testing can provide valuable information for a better EBV serology. However, because of technical difficulties, the use of avidity assays is generally very limited. EBV serology is traditionally based on IFA and several studies using IFA for EBV antibody avidity determination have been described previously [Gray and Wreghitt, 1989; de Ory et al., 1993; Andersson et al., 1994; Vetter et al., 1994]. Since quantitative IgG values are needed in order to calculate avidity indices, each sample has to be tested by IFA in several dilutions both with and without a urea washing step. This makes IFA avidity testing rather laborious. Furthermore, automation of IFA processing is not generally available, reading of the IFA slides tends to be subjective, and therefore, standardisation of IFA avidity is difficult to achieve. In contrast, EIA avidity testing offers the possibility of fully or par-

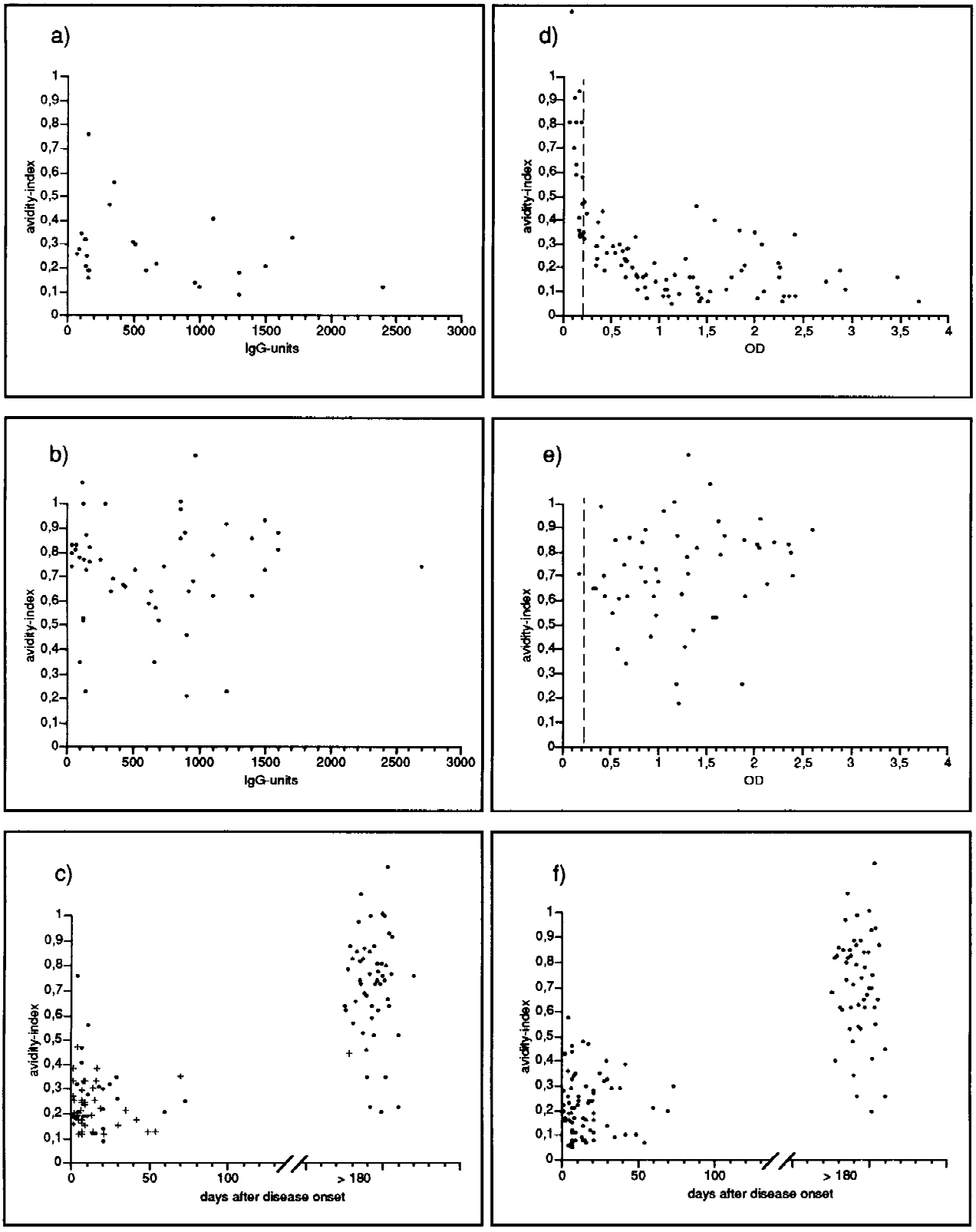

Fig. 3. Avidity determination with the VCA peptide EIA. IgG units without urea treatment and avidity indices of (a) acute infection samples and (b) past infection samples. (c) Time after onset of clinical symptoms and avidity indices based on IgG units; upper limit values for avidity indices are shown as crosses. OD values without urea treatment and avidity indices of (d) acute infection samples and (e) past infection samples. (f) Time after onset of clinical symptoms and avidity indices based on OD values.

tially automated processing of the microtiter plates and of objective avidity index calculation.

The use of a VCA IgG EIA for avidity determination has been described by Gray [1995] who used an assay based on purified EBV-infected cell lysate supplemented with purified EBV gp125. Avidity was calculated as the percentage reduction of OD values in the presence of 8 M urea and 50% reduction was considered to be the cut-off. Though all recent infection sera had low avidity and all past infection sera had high avidity in his study, the equivocal zone for the assay was between 41.47 and 57.4%. Since no information was given about the time span after disease onset, the kinetics of antibody maturation in his system are unclear.

In a previous comparison of EBV antibody avidity tests based on a VCA IFA and on an antigen mixture EIA both methods gave good results, but EIA avidity was easier to carry out and showed a better distinction between acute and past EBV infections [Schubert et al., 1996]. However, the EIA was less sensitive than the IFA for IgG detection early in the course of acute EBV infections. Several samples that were taken shortly after disease onset were positive with the antigen mixture IgM EIA and with the VCA IgG IFA, but negative with the antigen mixture IgG EIA. The early IgG response in primary EBV infections is mainly directed against VCA. Therefore, the composition of the antigen mixture, which includes VCA, EA, and EBNA, is an important determinant of the sensitivity of the assay for IgG detection in acute EBV infections. Since increased sensitivity for IgG is desirable for avidity calculations, it was attempted to establish an avidity assay based on a VCA IgG EIA.

The results of the comparison between the antigen mixture and the VCA peptide based avidity assays are presented in this study. The use of the antigen mixture assay for semi-automated avidity determination proved to be advantageous for several reasons. Firstly, while the sensitivity for detection of acute or recent EBV infections was very high with both assays, the specificity of the peptide assay was only 87%, making this test unsuitable as a confirmatory assay for resolving equivocal EBV serologies. The reason for this lack of specificity in avidity testing is unclear, but the antigen choice is likely to be a crucial parameter. The Sorin microtiter plates are coated with the synthetic p18-peptide of the VCA complex and the Behring microtiter plates are coated with a mixture of native antigens. It can be assumed that some epitopes which are recognized with high affinity on the native protein are not contained on the synthetic peptide. Therefore, native proteins may be superior to recombinant proteins for avidity testing. Secondly, in contrast to the VCA IgG IFA assay used previously, the VCA IgG EIA was considerably less sensitive than the antigen mixture EIA. Of the acute infection sera, 15 samples were negative for IgG with the peptide assay, but only 10 were negative with the antigen mixture assay. All these samples were positive for EBV IgM, showing that it is essential to carry out EBV IgM assays when an acute EBV infection is suspected and IgG is negative. Thirdly, using the starting dilutions recommeded by the manufacturers, the antigen mixture assay covers a larger range of IgG units (dilution 1:231; 25 U to approximately 2000 U) than the peptide assay (dilution 1:101; 20 U to 170 U). This is an important aspect for avidity assays. In initial testing with the peptide assay, about 30% of the samples had an IgG titer higher than the upper limit of the standard curve, making retesting at higher dilutions necessary. In contrast, no case had to be retested in higher dilutions with the antigen mixture EIA.

As mentioned above, IgG units of several serum samples in the acute infection group fell below the detection limit without or with the urea washing step in both assays. An avidity index based on IgG units could not be calculated in these cases. Since the starting dilution of the antigen mixture assay is 1:231, attempts were made to test negative samples at a lower dilution (1:55) in order to obtain IgG unit results which would allow to calculate an avidity index. However, this approach was successful for only a few samples. For the majority, IgG units remained below the detection limit without or with urea treatment even at lower dilutions. Furthermore, discrepancies between the results of the 1:231 and 1:55 dilution and a high variation at the lower dilution were observed on repeat testing. Therefore, it was decided not to use lower dilutions than those recommended by the manufacturers.

Several consecutive serum samples of patients with acute EBV infection were examined. In all cases, the avidity index increased with time. One patient in group 1 had a high avidity index 10 weeks after disease onset (0.66). Since samples of the same patient taken at day 5 and 29 were found to have low EBV IgG aviditiy, it was assumed that the antibody response had matured to be of high avidity within 10 weeks in this patient and therefore, the 0.66 index was not considered to be a false negative result in the statistical analysis. Although the number of acute infection samples taken at more than four weeks after disease onset was small in this study, it appears that low avidity antibodies can be detected by this test system as late as two months after disease onset. This observation is in contrast to the data of Andersson et al. [1994], who reported a significant rise in EBV VCA antibody avidity after only three weeks. Since avidity testing is a complex process and the avidity index as a measurement of antibody maturity is influenced by numerous variables which have not yet been standardized, there are several explanations for this discrepancy, among them being the urea concentration, the urea incubation period, the antigen preparation, the assay reproducibility and the cut-off definition. It is likely that any deductions made about the kinetics of antibody maturation are highly dependent on the assay used. Because knowledge of the kinetics is important for the clinical interpretation of avidity results, further avidity studies on follow-up serum samples of patients with acute EBV infections have to be performed with the antigen mixture assay.

Using OD values instead of IgG units presents a more simplified approach to calculate avidity indices, but to obtain a high sensitivity for diagnosing acute or recent EBV infections with the antigen mixture assay, it is essential to calculate avidity indices based on one-point-quantification units. This has the added advantage that two results are produced by the avidity assay: a quantitative EBV IgG value and the avidity index. For the peptide assay, no increase in sensitivity was seen when standard curve derived IgG units were used instead of OD values.

Even though there was no overlap between the avidity indices of acute and past infections in this study when the antigen mixture assay and one-point-quantification units where used, a theoretical equivocal zone remains. It can be calculated from the mean values (MV) and standard deviations (SD) of the avidity indices shown in Table 1. The equivocal zone includes all values between past infection MV−3*SD and acute infection MV+3*SD. For the antigen mixture assay, the equivocal zone is 0.47 to 0.53 with IgG units and 0.61 to 0.72 with OD values. Avidity indices within this range have to be considered as indeterminate at present. Further studies on a larger group of samples are needed in order to verify that these equivocal zones are appropriate.

Automation of serological tests offers the advantage of improved reproducibility and cost effectiveness compared to manual testing. Thus, in order to increase the acceptance of avidity assays in routine laboratories, automation is important. In this study, all processing steps of the microtiter plates were performed automatically except for the urea washings. The danger of crystallization of the high molar urea solution in the washer tubing and the complicated urea washing procedure are the main obstacles of full automation. Studies with other denaturing agents that pose less practical problems are under way.

It is important to note that the EIA used in this study are not intended for avidity testing. Instead, they are part of different strategies for a comprehensive EBV serology as recommended by the manufacturers. It was not the aim of this study to evaluate these different strategies, and deductions on the performance of the EBV IgG EIA in the context of their intended purpose should not be drawn from the avidity results.

In conclusion, it was demonstrated that a partially automated avidity test based on a commercially available EBV IgG EIA can distinguish acute and past EBV infections with very high sensitivity and specificity. The antigen choice seems crucial in establishing clinically useful avidity assays. Denaturing agents other than urea may facilitate a full automation of avidity testing in the future. Avidity tests are an important additional marker, that can be used routinely to confirm or exclude acute EBV infections.

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